Supplementary Material

Taghon, Yui, and Rothenberg: *Mast cell diversion of T-lineage precursor cells by the essential T-lineage transcription factor GATA-3*

Supplemental Table

Supplemental Figures 1-6

Supplemental Table 1: New primer sequences for Q-RT-PCR

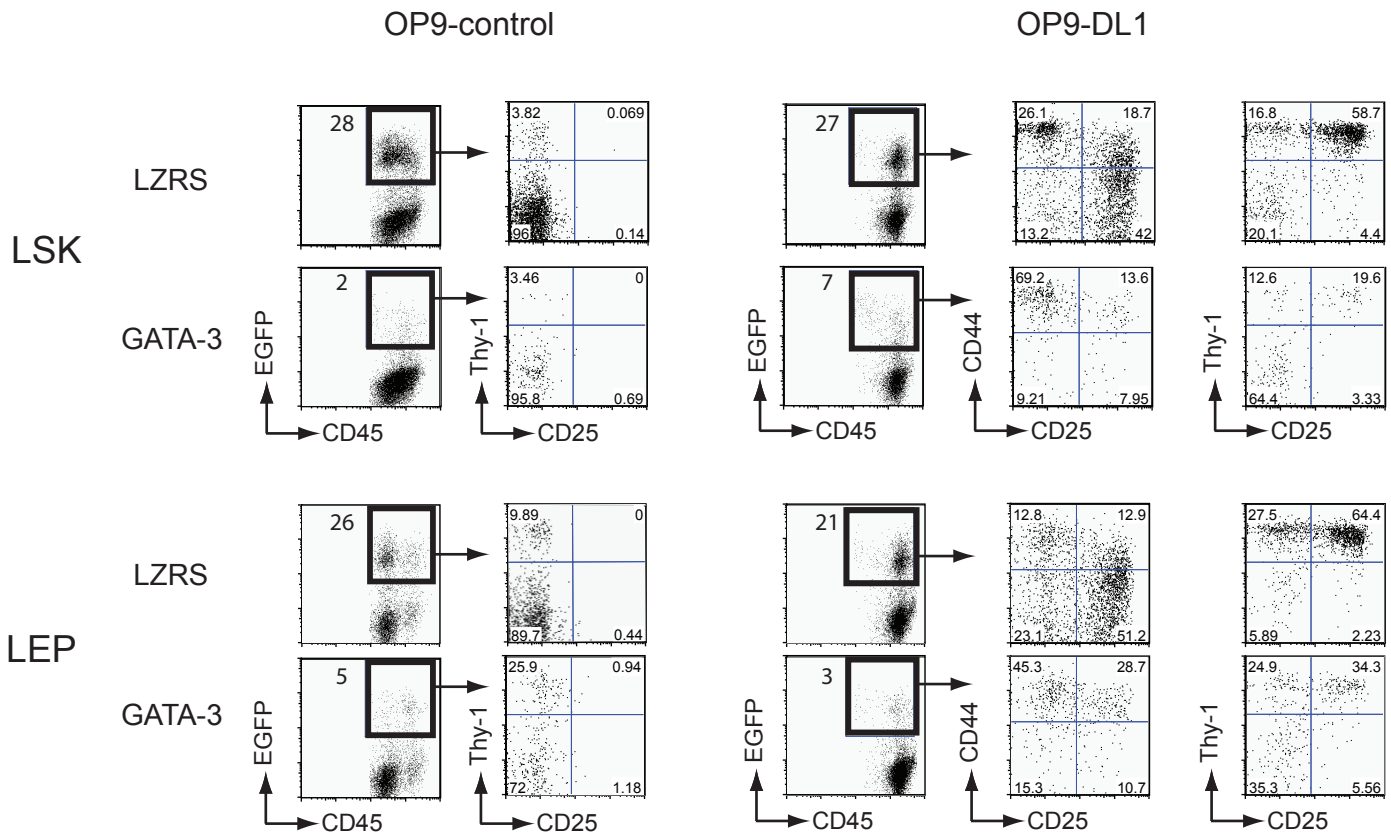
Gene	Forward Seq.	Reverse Seq.
β-actin	ACACCCGCCACCAGTTC	TACAGCCCGGGGAGCAT
Bcl11b	GGGCGATGCCAGAATAGAT	GGTAGCCTCCACATGGTCAG
CPA3	GACCATCCAGTCAACCTTGG	CTGCCTGCGATTTCATCTTT
EPO-R	CTGTTGCTGACGGTTCTGG	AGCAGCCACAGCTGGAAGT
Gata3 all	CCTGCGGACTCTACCATAAAA	GTGGTGGTGGTCTGACAGTTC
Hemoglobin-b1 (Hbb-b1)	ATGGCCTGAATCACTTGGAC	ACGATCATATTGCCCAGGAG
Hes1	CCAAGCTAGAGAAGGCAGACA	CGGTATTTCCCCAACACG
Mitf	CCCCAAGTCAAATGATCCAG	CCTTAGCTCGTTGCTGTTCC
Perforin	CCAATTTTGCAGCTGAGAAGAC	CGCCTTTTTGAAGTCAAGGTG
Sox4	TCAAGGACAGCGACAAGATTC	GCCGGTACTTGTAGTCAGGGTA
TPO-R/c-mpl	CTGCTAAAGTGGCAATTTCCTG	GACTTAGGGCTGCAGTGTCTCT
Tryptase/Mcpt6	GGAGGACATGAGGCTTCTGA	GGGCTTTTGATGTGCGGT

Supplementary Figure 1

Inhibition of T-cell lineage specification of prethymic precursors by forced expression of GATA-3.

Transduction of wild-type BDF1 LSK cells or LEPs with GATA-3 (GATA-3) fails to induce $CD25^{+}Thy\text{-}1^{+}$ T-lineage specified cells in the absence of Notch signaling (OP9-control cultures), and inhibits the generation of $CD25^{+}$ DN2 and DN3 cells in the presence of Notch signaling (OP9-DL1 cultures) as compared to empty vector control transductants (LZRS). This block is independent of the absence (this figure) or presence (Fig. 1) of constitutive expression of a Bcl2 transgene. Cultures were analyzed by flow cytometry after 5 days of OP9-control and 5 days of OP9-DL1 coculture with markers as indicated.

Supplementary Figure 1



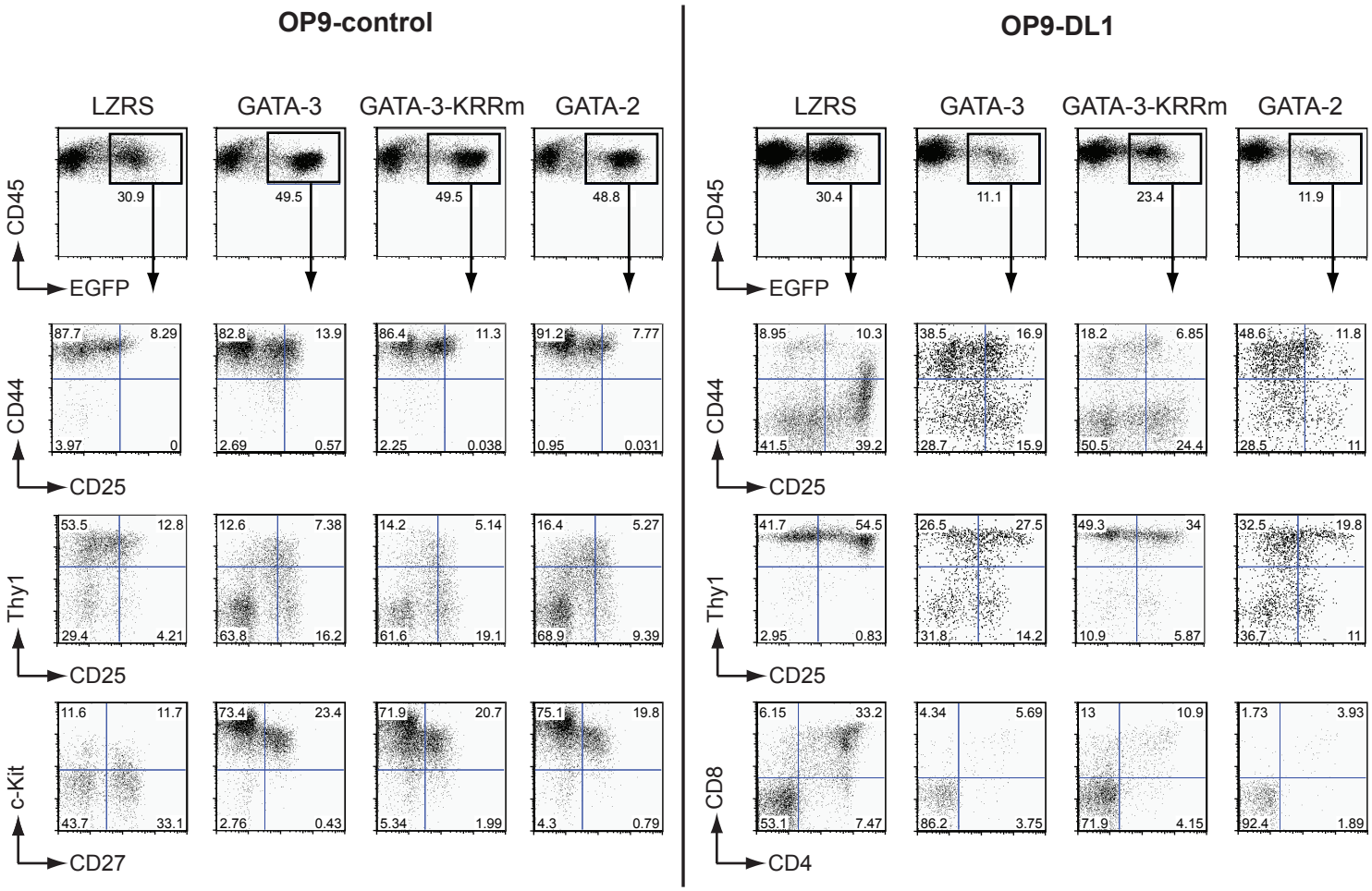
Supplementary Figure 2

Stage-specific proliferative and differentiative effects of GATA-3 and GATA-2: T-lineage inhibition but not mast-cell differentiation depends on the KRR motif.

Comparison of effects of transduction with GATA-3, GATA-2, and the KRR mutant of GATA-3. Bcl2-tg thymocytes were infected overnight and then transferred to OP9-control and OP9-DL1 stromal cells. Cultures were analyzed after 6 days on OP9-control and 5 days on OP9-DL1 by flow cytometry with the indicated markers. On OP9-control stroma (left panels), GATA-3-KRRm and GATA-2, like GATA-3, redirect fetal thymocytes to generate a population of c-Kit^{++} thymocytes on OP9-control stromal cells (lower left). However, on OP9-DL1 stroma (right panels), GATA-3-KRRm allows better T-cell survival (top panels, GFP+ CD45+ cell recovery) and better T-lineage differentiative progression (more CD44^{-} CD25^{-} DN4 cells, second row; more CD4^{+} CD8^{+} DP cells, bottom row) than wild-type GATA-3 or GATA-2. Wildtype GATA-3 and GATA-2 inhibit viability less with these Bcl2-transgenic cells than with normal, nontransgenic thymocytes, but these samples provide enough viable cells to provide a better comparison of differentiative effects.

Supplementary Figure 2

Bcl2-tg fetal thymocytes



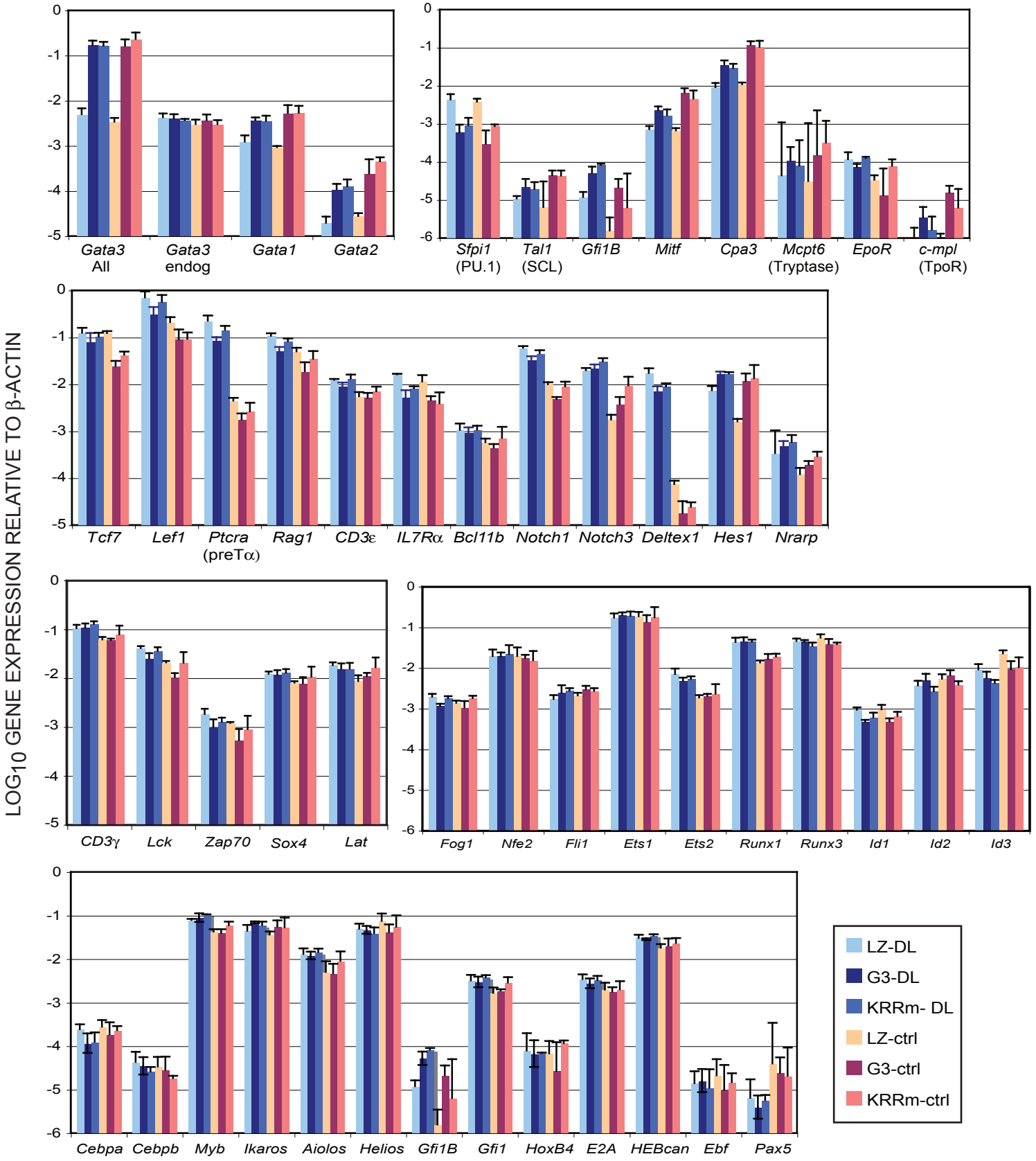
Supplementary Figure 3

Additional quantitative real-time RT-PCR analysis of sorted fetal thymocytes at an early timepoint after transduction with LZRS, GATA-3 or GATA-3-KRRm.

Samples are the same as those used for Fig. 4b, along with samples transduced in parallel with GATA-3-KRRm. Cells were sorted as GFP^+ $CD45^+$ after overnight infection and 1 day of coculture with OP9-control or OP9-DL1 (~40 hr overall). Expression levels for all genes are presented in units relative to β-actin. Data shown are the averages of 4 samples derived from two different culture conditions in two independent experiments (examples shown in Supplementary Fig. 4), with error bars indicating standard deviations. Note the selectivity of the gene expression effects and the general independence of GATA-3 and DL1-dependent effects. Also note that KRRm is as potent as wildtype GATA-3 in most positive regulatory effects, but tends to differ by showing weaker repression of pro-T cell genes, consistent with its milder impact on T-cell development.

Supplementary Figure 3

Q-PCR ANALYSIS OF 40 H SORTED CELLS FROM OP9 CO-CULTURE

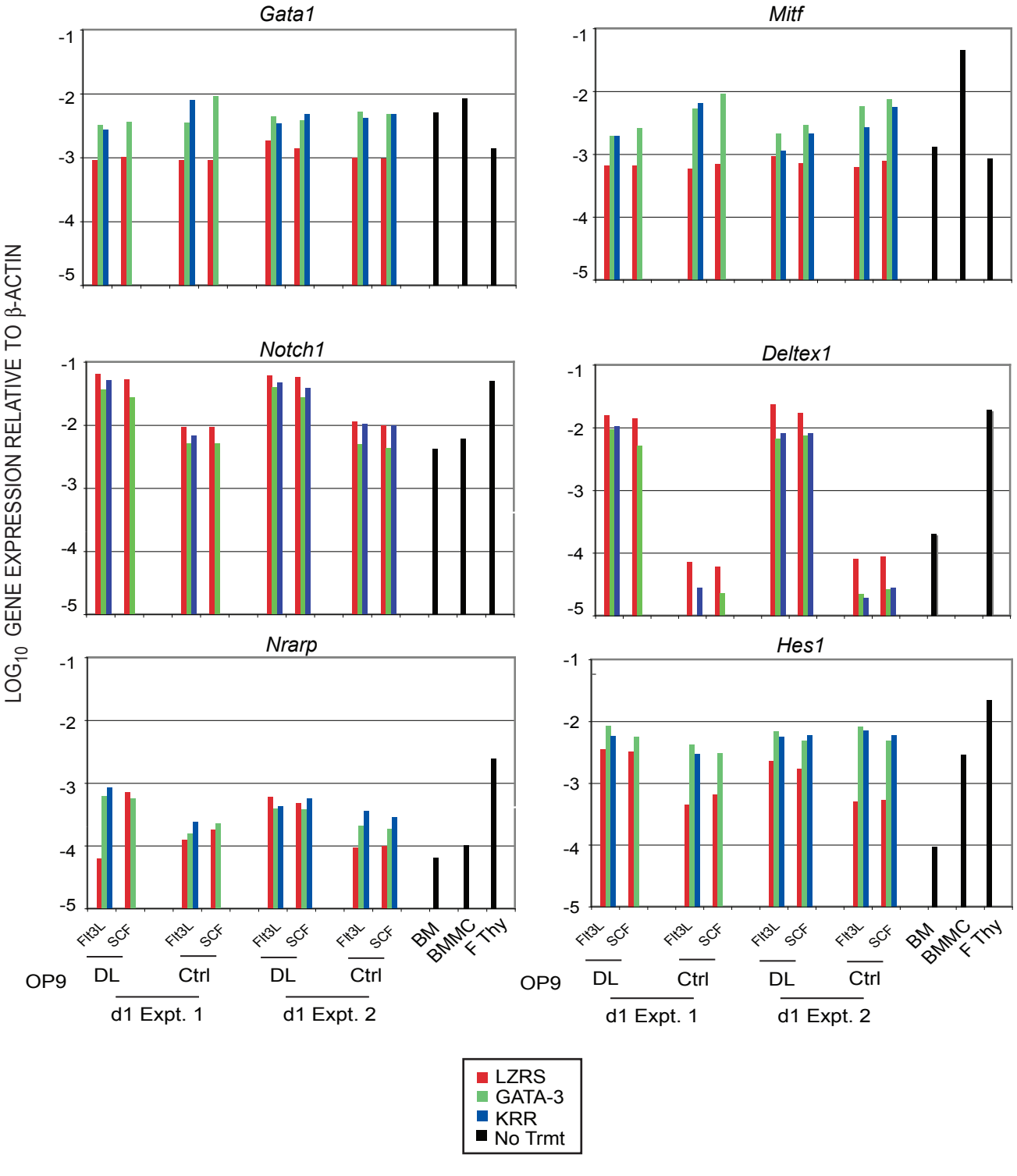


Supplementary Figure 4

Early effects of GATA-3 and Notch-signals on Notch-responsive genes and mast-cell genes, in the presence and absence of SCF.

Quantitative real-time RT-PCR analysis of sorted thymocytes transduced with LZRS (red), GATA-3 (green) or GATA-3-KRRm (blue), sorted as GFP^+ $CD45^+$ after overnight infection and 1 day of coculture on OP9-control or OP9-DL1. Individual sample measurements are shown for two representative mast-cell genes, *Gata1* and *Mitf*, and four Notch-responsive genes, *Notch1*, *Deltex1*, *Nrarp*, and *Hes1*, in the four samples that are averaged to generate the data in Fig. 4b and SFig. 3. Results from two independent experiments are shown. Cultures were set up for each vector/OP9 combination with 5 ng/ml IL7 plus either 5 ng/ml Flt3L or 5 ng/ml SCF, as indicated. cDNA from unmanipulated bone marrow cells (BM), bone marrow cultured mast cells (BMMC), and fetal thymocytes (F Thy) are shown for reference (black bars). Expression levels for all genes are presented in units relative to β-actin using the ΔCt method as in Fig. 4c and SFigs.3 and 5. Data shown for each cDNA sample is the average of three replicate PCR samples. Note the lack of any systematic difference between samples cultured with Flt3L + IL-7 and those cultured with SCF + IL-7 at this time point. Results show that GATA-3 overexpression has distinct effects on different Notch target genes, ranging from repressive effects on *Deltex1* to stimulatory effects on *Hes1*.

SUPPLEMENTARY FIGURE 4



Supplementary Figure 5

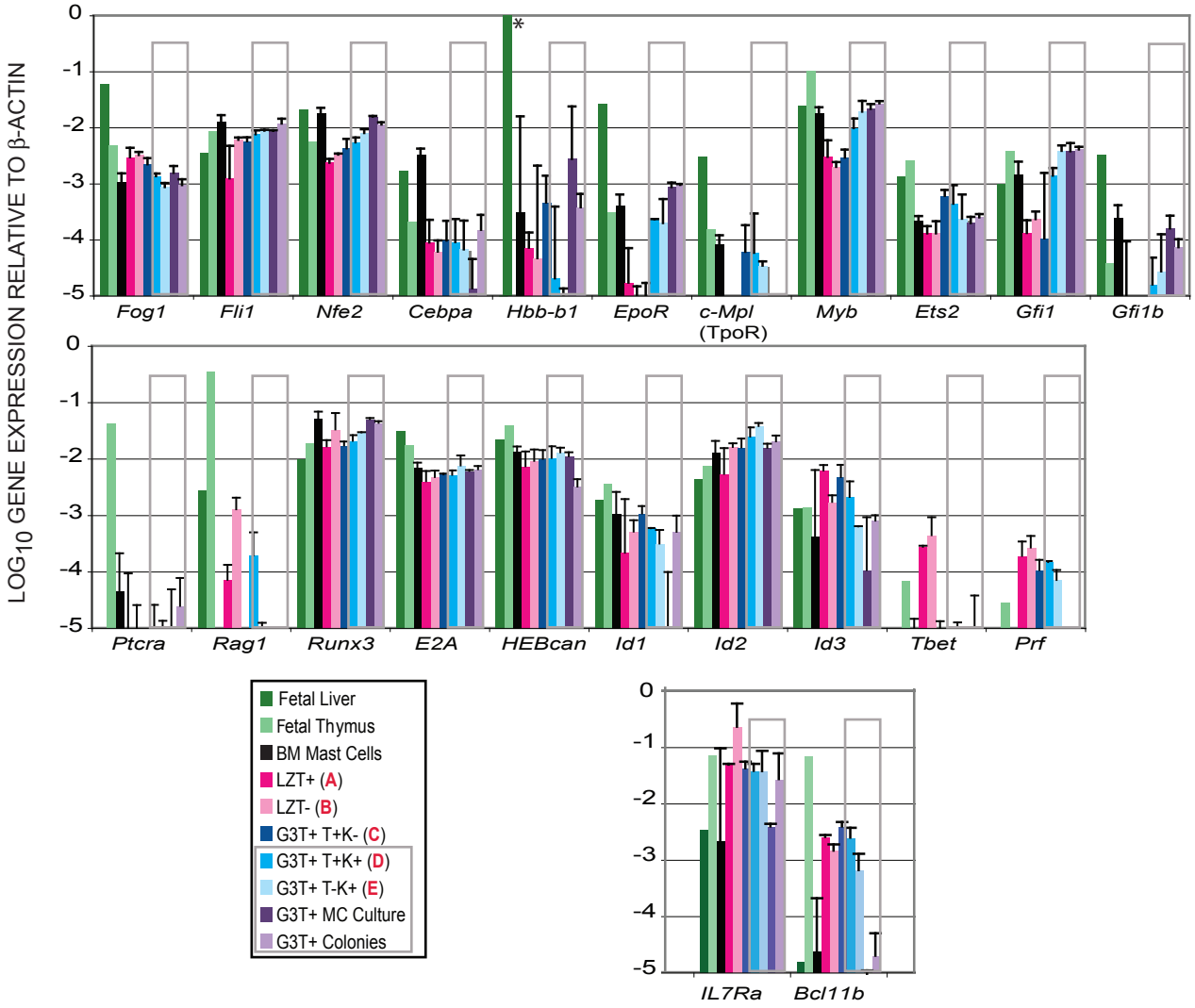
Additional gene expression effects by GATA-3 during redirection of fetal thymocytes to the mast-cell lineage

Quantitative real-time RT-PCR analysis on additional genes, using the same samples after 5 days of culture on OP9-control cells, as shown in Fig.4c. The upper panel shows effects on genes canonical for diverse hematopoietic fates in addition to T and mast-cell development. The lower panels show results for genes involved specifically in pro-T cell development. Note that at this time point, the prolonged withdrawal of Notch/Delta signaling has severe adverse effects on certain pro-T cell genes such as *Ptcra* (encoding preTα) and *Rag1*, irrespective of the presence of GATA-3. Expression levels for all genes are presented in units relative to β-actin, calculated by the ΔC_T method. Data shown are the average of two independent experiments with error bars indicating one standard deviation. Results emphasize the good match between BMMC (black bars) and GATA-3-transduced fetal thymocytes capable of generating mast cells (samples within gray boxes) in gene expression profiles, while disfavoring alternative possibilities that the c-Kit^{++} cells might be of erythromegakaryocytic, C/EBPα^{+} granulocyte, or natural killer lineages.

* Expression of hemoglobin β (Hbb-b1) measured in the fetal liver control was beyond the scale shown (~15 units relative to β-actin). Expression in the crude fetal thymus sample (not shown) was also high, presumably due to blood contamination, while the level measured in purified DN3 pro-T cells in this assay was 0.02 relative to β-actin (not shown). By comparison, these results emphasize the lack of globin expression in the mast cells and in the GATA-3 transduced fetal thymocytes.

Supplementary Figure 5

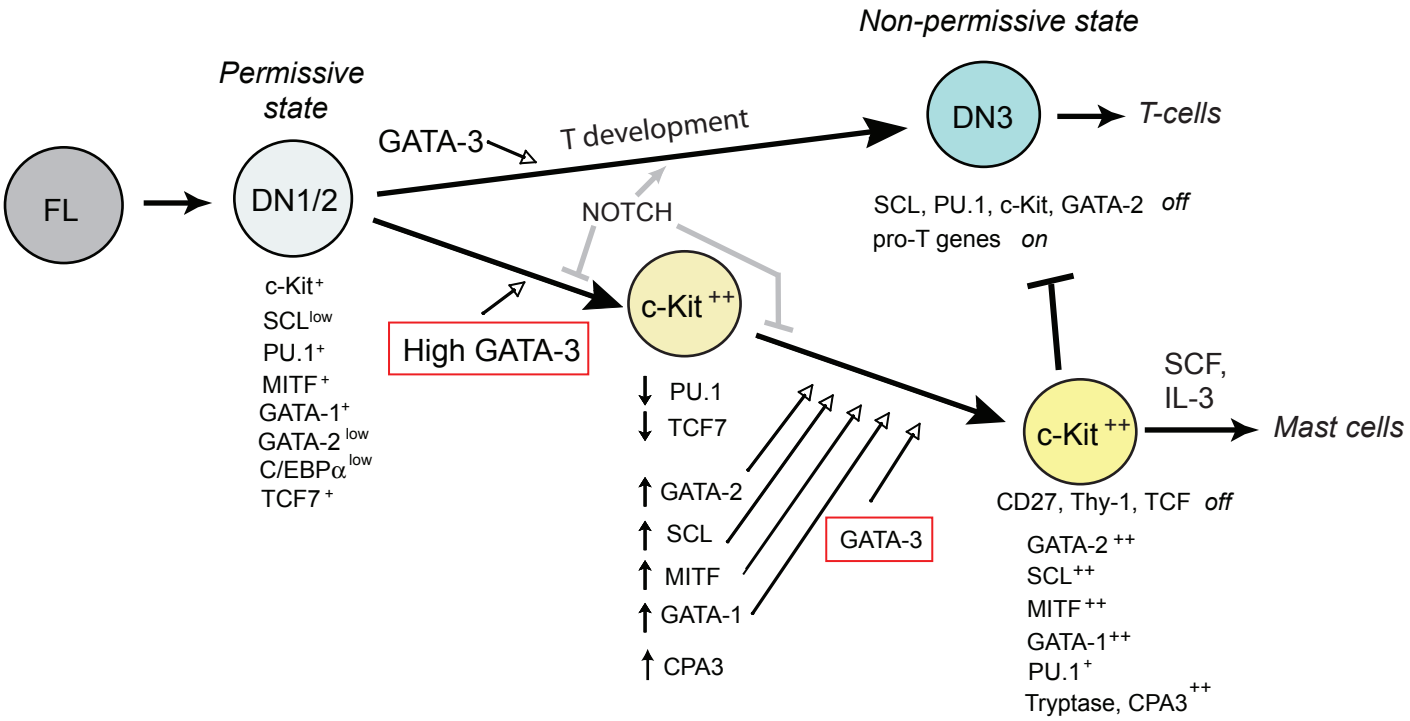
Q-PCR ANALYSIS OF CELLS SORTED AFTER 5 DAYS IN OP9-CONTROL OR LONG-TERM CULTURE



Supplementary Figure 6

Regulatory pathway for pro-T cell diversion to mast cells.

Summary of data from Figs. 4 and 5, presented in a developmental framework. Baseline gene expression differences between populations are from refs 22, 23, and M. A. Yui, unpublished results. Proposed sites of action of Notch signaling are based on effects shown in Figs. 2 and 4, and SFigs. 3-5. For discussion, see text.


Non-permissive state
Permissive state
GATA-3
T development
DN3
T-cells
FL
DN1/2
NOTCH
SCL, PU.1, c-Kit, GATA-2 off
pro-T genes on
c-Kit+
SCLlow
PU.1+
MITF+
GATA-1+
GATA-2 low
C/EBPα low
TCF7+
High GATA-3
c-Kit ++
PU.1
TCF7
GATA-2
SCL
MITF
GATA-1
CPA3
GATA-3
SCF,
IL-3
c-Kit ++
Mast cells
CD27, Thy-1, TCF off
GATA-2 ++
SCL++
MITF++
GATA-1++
PU.1+
Tryptase, CPA3 ++

Suppl Fig. 6